Poster II-7

P21 Rho GTPase Activity Automatically Correlated to Cell Motility Using Digital Microscopy

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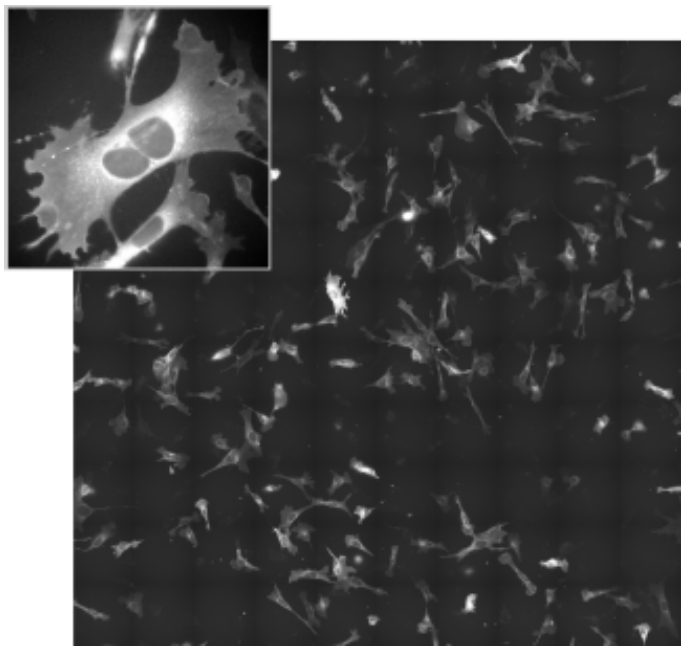
RhoA GTPase is thought to be very important in controlling cell motility. However, many conclusions regarding these proteins are based on subjective observations of single cell micrographs, making statistical correlation of activity-function impossible. We have developed automated, high-throughput, digital biological tools to directly measure the activation of this important signaling protein over populations of both fixed and living cells. These microscopy tools enabled rapid scoring of hundreds of cells moving over a fibronectin substrate. The measurements were performed on mouse embryonic fibroblasts with our recently developed single-chain FRET biosensor that reports the activation of RhoA GTPase. Measurements of the intracellular distribution of RhoA activity in hundreds of cells per experiment were automated on an adaptation of the Q3DM Inc. Eidaq 100 using: fast autofocus, 12-bit image acquisition, a segmentation algorithm based on K-mean clustering for separation of the background and the foreground information in two fluorescence channels of the same cells of interest, image registration using cross-correlational alignment of the segmented binary images, and calculation of the ratiometric images. In the figure below, an example of 10 x 10 combined fields of view in the FRET channel of mouse embryonic fibroblasts expressing RhoA biosensor, under 40x magnification (N/A 1.3 oil immersion) is shown. Each field is acquired at full 12-bit depth and the inset shows a blowup of one representative field. The analyses included determination of a cell shape factor that transposed complex cell morphology into simple 2 dimensional plots for quantitatively categorizing populations of cells based on the cell shape. Fitting a triangle to the cell segments automated polarization angle measurements that correlated with the degree of Rho activation in different parts of cells. We have classified activation patterns of Rho based on cell shape and polarization to extract data correlating cell motility and direction of cell motion to Rho activity. Our results indicate distinct intracellular localization patterns of Rho activation, prevalent in the direction of protrusions at the cell periphery. We have consistently observed Rho activations in areas of cells just prior to formation of and within large protrusions. Results from fixed cell assays were compared to our multi-field, live-cell tracking data to validate the index-based correlation of Rho activity, cell shape, polarization and movement direction. The combination of these automated digital biology tools – high-throughput microscopy and novel Rho GTPase biosensors – opens the door to exciting direct studies, of cell behavior with statistically relevant activity-function information in large populations of cells.



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